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Efficacy of Newborn Bovine DNA Samples Taken Via Different Mediums in Assigning Paternity

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Summary

DNA samples from 25 newborn calves taken via hair, ear notch, and nasal swabs were used to determine the efficacy of sampling method in assigning parentage. Nasal swab samples were collected at six time points from birth to 120 hours post-birth. Calf samples and all candidate sires were genotyped with a 99 SNP parentage panel. Nasal swab collection time did not result in significant differences in the ability to assign the correct sire, although differences were seen in apparent cleanliness of the sample. Clean nasal swab samples are comparable in efficacy to hair and ear notch samples in assigning parentage.

Introduction

It is possible to extract DNA from multiple tissues including hair follicles, semen, blood, or nasal swabs. A preferred procedure for collecting DNA is one in which labor and cost are minimized and consistent high-quality genotypes are produced. Nasal swab samples are desirable because they require less labor and are an easier method for laypeople to use as compared to other procedures. The objectives of the study were to determine if nasal swabs (NS) were viable sources of DNA for paternity assignment in newborn calves and to determine how they compared to other standard sample types such as hair follicles (HF) and ear notches (EN).

Procedure

Tissue samples were obtained from randomly selected calves (n=25) and all potential candidate sires (n=8) from the University of Nebraska–Lincoln Teaching Herd. Three sample types were taken from each calf including HF, EN, and NS. At birth each calf had 25 to 30 HF taken from the tail switch and an EN sample taken from the tip of the ear using an appropriate sized ear notcher. Upon removal, hair follicles were placed on the hair card adhesive strip, put in a plastic bag and stored at room temperature (GeneSeek, Lincoln, Neb.). Once the EN was collected it was placed in a 2.0 ml plastic tube and stored at -20 C°. Nasal swab samples were obtained via a tube with stabilizer solution and a cotton swab attached to the outside of the cap. After gently swabbing the inside of a calf's nasal cavity with the cotton swab, the cap was unscrewed, inverted, and re-screwed so the cotton swab was inside the tube and submerged in the stabilizer solution (DNA Genotek Inc., Kanata, Ontario Canada). Nasal swab samples were taken at six different time points from each calf including 0 (birth), 6, 12, 24, 72, and 120 hours post birth and stored at room temperature. A categorical scale from 1 to 3 was used to assign a cleanliness score to each NS sample where 1 was defined as extremely clean and 3 as extremely dirty. DNA was extracted from the nasal swab sample using Promega's MegaZorb DNA mini prep kit protocol (DNA Genotek Inc., Kanata, Ontario Canada). DNA samples from candidate sires were obtained from semen samples. Semen samples have been shown to be a robust DNA collection technique. In the current study, the average (SD) percentage of SNP called was 89.6 (All DNA samples were genotyped at GeneSeek with a commercially available single nucleotide polymorphism (SNP) parentage panel that contained 99 highly polymorphic SNP.

Data edits were performed to remove samples that did not yield any genotypes (n=1 NS at hour 120), true sire could not be determined (n= 1 animal), and abnormally low SNP call rates (n= 1 EN). Missing data occurred for four NS due to one calf mortality before hour 12. The numbers of observations in the final analysis are described in Table 1.

The efficacy of the NS as a DNA sampling technique was evaluated based on the total number of SNP genotypes called and the number of exclusions (disagreement between SNP genotypes) between both the true sire and EN from the same animal. The number of exclusions was determined using SireMatch. Statistical analysis of differences in the number of exclusions for all sample types was performed by fitting two models, one for exclusions from the true sire (included EN, HF, and all NS samples)

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Table 1. Least-squares (LS) mean number of exclusions from the true sire and ear notch between all sample types.

	Exclusions from True Sire			Exclusions from Ear Notch		
Sample Type	N	LS Mean	Standard Error	N	LS Mean	Standard Error
Nasal Swab, hour 0	24	0.125	0.160	24	0.125	0.133
Nasal Swab, hour 6	24	0.083[a]	0.160	24	0.083	0.133
Nasal Swab, hour 12	23	0.043[a]	0.164	23	0.087	0.134
Nasal Swab, hour 24	23	0.174	0.164	23	0.087	0.134
Nasal Swab, hour 72	23	0.043[a]	0.164	23	0.087	0.134
Nasal Swab, hour 120	22	0.045[a]	0.168	22	0.046[a]	0.136
Hair	24	0.083[a]	0.160	24	0.375[b]	0.133
Ear Notch	24	0.458[b]	0.160	—	—	—

[a,b]Least-squares means with different superscripts within a column differ ($P < 0.10$).

and another for exclusions from the EN (included HF and all NS samples), which included the fixed effect of sample type. Statistical analysis of differences in the number of exclusions by NS collection time was performed by fitting a model for exclusions from the true sire (included all NS samples) that included fixed effects of NS collection time and cleanliness score. An exclusion can occur if the candidate sire is in fact not the true sire of the calf, or if the candidate sire is the true sire of the calf but either the sire or calf was mis-genotyped for a particular SNP giving rise to an incorrect SNP genotype being assigned ("missed call").

The true sire of each calf was determined by calculating the number of exclusions between each sire and each calf sample (HF, EN, and all NS samples). The true sire was then assigned to each calf if the majority of sample types had no more than one exclusion from a given sire.

Results

The least-squares (LS) mean numbers of exclusions by sample type are presented in Table 1. Sample type (HF, EN, and all NS samples) did not have a significant effect on the number of exclusions from the true sire (P=0.63) or EN (P=0.65). The mean number of exclusions from the true sire for HF and NS samples at time points 6, 12, 72, and 120 hours tended (P= 0.07 to 0.09) to be lower than EN samples.

Table 2. Least-squares (LS) mean number of exclusions from the true sire between cleanliness score.

	Exclusions From True Sire	
Cleanliness Score[2]	LS Mean[1]	Standard Error
1	0.017[a]	0.041
2	0.124[b]	0.039
3	0.182[b]	0.073

[1]Each least-squares mean has been adjusted for nasal swab collection time.
[2]A score of 1 being the cleanest and 3 being the dirtiest.
[a,b]Least-squares means with different superscripts within a column differ ($P < 0.10$).

This was unexpected as the hypothesis was that EN samples would prove to be the most robust sample type, and HF would perform the worst. In the current study, the hair sample and NS technique utilized during the experiment proved to be quite reliable, with 100 and 99.2 percent of the hair and NS samples, respectively, having less than two exclusions from the true sire. However, caution should be used when taking hair samples from young calves to ensure that enough fully formed hair follicles are obtained.

NS collection time did not have a significant effect on number of exclusions from the true sire (P=0.58). The cleanliness score tended to have a significant effect (P= 0.066) on the number of exclusions from the true sire. The LS mean numbers of exclusions from the true sire for each cleanliness score are presented in Table 2, which illustrate that as the cleanliness score increases (i.e., samples become dirtier), the number of exclusions from the true sire increases. Thus, swabbing an extremely dirty nasal cavity may lead to poorer quality DNA and an increase in the number of exclusions from the true sire. In the current project only 8 of the 146 NS samples had call rates less than 70%, and 107 of the 146 had call rates greater than 90%.

The time at which the NS sample was taken from a calf within the first 120 hours after birth did not significantly impact the number of exclusions from the true sire. However, the cleanliness of the sample did affect the number of exclusions, or missed calls, between an NS sample and the calf's true sire. Thus, while the quality of DNA obtained from the NS is vital to its efficacy, the time the swab is taken on a newborn or the type of sample (i.e., HF, NS, EN) seems irrelevant to assigning parentage. Consequently, the choice of sample type should be determined by cost of sample collectors and ease of collection.

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